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Distinct Processes Drive Diversification in Different Clades of Gesneriaceae

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Abstract.—Using a time-calibrated phylogenetic hypothesis including 768 Gesneriaceae species (out of ~3300 species) and more than 29,000 aligned bases from 26 gene regions, we test Gesneriaceae for diversification rate shifts and the possible proximal drivers of these shifts: geographic distributions, growth forms, and pollination syndromes. Bayesian Analysis of Macroevolutionary Mixtures analyses found five significant rate shifts in Beslerieae, core *Nematanthus*, core Columneinae, core *Streptocarpus*, and Pacific *Cyrtandra*. These rate shifts correspond with shifts in diversification rates, as inferred by Binary State Speciation and Extinction Model and Geographic State Speciation and Extinction model, associated with hummingbird pollination, epiphytism, unifoliate growth, and geographic area. Our results suggest that diversification processes are extremely variable across Gesneriaceae clades with different combinations of characters influencing diversification rates in different clades. Diversification patterns between New and Old World lineages show dramatic differences, suggesting that the processes of diversification in Gesneriaceae are very different in these two geographic regions. [Diversification rates; epiphytism; Gesneriaceae; historical biogeography; Lamiales; pollination syndrome.]

An overarching goal of phylogenetic systematics is to understand both the patterns of diversification of organismal lineages and the processes by which these patterns are formed (e.g., Rabosky et al. 2013; Cornwell et al. 2014). Methodological advances, both in terms of data acquisition and analytical ability, have changed considerably over the past 10 years, and now it is common to have hundreds to thousands of species and numerous DNA sequence regions included in phylogenetic analyses together (Smith S.A. et al. 2009; Hinchliff and Roalson 2013; Zanne et al. 2014; Cornwell et al. 2014). Analytical advances have been driven by an interesting combination of increased computational speeds, novel algorithmic approaches to phylogenetic analyses, and a large focus on new analytical approaches to hypothesis testing, often in the R statistical computing platform (e.g., R Development Core Team 2011; Matzke 2013a, 2013b; Ng and Smith 2014).

These advances now allow for the integrated study of multiple patterns and processes on large phylogenetic scales to more directly address fundamental questions of lineage diversification (Rabosky et al. 2013; Zanne et al. 2014; Cornwell et al. 2014; Schwery et al. 2015). Here, we apply an integrated approach to understand the drivers of diversification in the tropical flowering plant lineage Gesneriaceae. Gesneriaceae (African violets, gesneriads) is a Lamiales lineage of approximately 160 genera and approximately 3300+ species, including perennial herbs, shrubs, and small trees (Möller and Clark 2013; Weber et al. 2013). After the divergence of the monotypic Andean genus *Sanango* from the rest of Gesneriaceae, the family split into two lineages: a predominantly New World Gesnerioideae (1200+ species) and a predominantly Old World Didymocarpoideae (2100+ species; Möller and Clark 2013; Weber et al. 2013). Gesneriaceae are particularly diverse in their flower morphology, and have well-documented cases of convergence and parallelisms in pollination syndromes (Harrison et al. 1999; Roalson et al. 2003; 2005; Perret et al. 2007; Clark et al. 2011). This has lead several authors to consider diversification of floral form to be one of the critical driving factors of lineage diversification in the gesneriads (Martén-Rodríguez et al. 2009; Perret et al. 2013). However, this has yet to be tested in a rigorous way across clades of any size in Gesneriaceae. Between the Old and New Worlds, there are significant differences in what pollinator lineages are available, particularly the availability of hummingbirds in the New World but not the Old, and it is possible that this might differentially affect changes in floral form and pollinator specialization in these clades. Further, it has been suggested that there are broad-scale differences in speciation and extinction rates between the New and Old World (Antonelli et al. 2015), and Gesneriaceae provide comparable sister clades to assess this hypothesis.

Epiphytism is a well-known habit specialization that is found in many plant lineages, but best studied in Orchidaceae, Bromeliaceae, Cactaceae, and Gesneriaceae (Gravendeel et al. 2004; Silvera et al. 2009; Calvente et al. 2011; Givnish et al. 2014). Some have suggested that epiphytism opens new habitat niches and can increase rates of diversification (Givnish 2010; Givnish et al. 2014, 2015), but it is yet unclear whether this is the case across epiphytic lineages, most of which have yet to be studied in terms of diversification rate. This growth form has originated multiple times in Gesneriaceae, but is most prominent in the predominantly South American clade Columneinae (Weber et al. 2013).

Additional factors have been suggested to be important to diversification in gesneriads, including diversity of vegetative growth forms (particularly unifoliate plants) in the *Streptocarpus* clade (Möller and Cronk 2001), and adaptation for dispersal across islands in the Pacific in *Cyrtandra* (Atkins et al. 2001; Clark

et al. 2009). Geographic and geological influences on diversification also have been invoked for some clades (Perret et al. 2007; Dimitrov et al. 2012). While all of these characteristics have been studied to some degree in small-scale studies of each of these individual clades, they have not been studied in the larger context of how they might influence differences in diversification rates among clades, and whether particular characters are contributing more or less to these processes.

Here, we use a mega-phylogeny approach coupled with assessment of patterns of rate variation in a Bayesian framework (as implemented in Bayesian Analysis of Macroevolutionary Mixtures [BAMM]), rate variation of morphological characters in a likelihood framework (as implemented in Diversitree), and influence of geography on diversification rates (as inferred by Geographic State Speciation and Extinction model [GeoSSE]) to test the influence of morphology and geography on diversification patterns across Gesneriaceae. We will specifically address the following questions: 1) Is there support for different rates of diversification in different clades of Gesneriaceae? 2) What influence, if any, do geographic, floral, and growth form characteristics of interest play in diversification rates? 3) Do we see different patterns between the New World and Old World clades of Gesneriaceae?

Materials and Methods

Taxon Sampling

The subfamilies and tribes of Gesneriaceae have been the focus of many targeted phylogenetic analyses; hence, there are many available sequences from Gesneriaceae taxa (Atkins et al. 2001; Zimmer et al. 2002; Roalson et al. 2005, 2008; Clark et al. 2006, 2009, 2011, 2012; Roalson and Clark 2006; Perret et al. 2007, 2013; Möller et al. 2009, 2011; Wang et al. 2010; Puglisi et al. 2011; Weber et al. 2011; Woo et al. 2011; Smith and Clark 2013; among others, for a review see Möller and Clark 2013). To investigate phylogenetic relationships in Gesneriaceae, we used PhyLoTA (Sanderson et al. 2008) to assemble a data set of nucleotide sequences available from GenBank release 194.0 (Supplementary Appendix 1, available on Dryad at http://dx.doi.org/10.5061/dryad.1br13). We downloaded nucleotide sequences from plastid, mitochondrial, and nuclear loci in orthologous gene clusters containing at least 10 species. Orthologous gene clusters were identified by all-against-all BLAST searches (*E*-value = 1e−01, > 51% coverage) followed by single-linkage clustering. For each cluster, we removed sequences that did not have a proper species name, removed any subspecies designations, and removed all but one sequence per species. Voucher specimens for sequences obtained from GenBank were not examined for verification of identifications.

These clusters were then aligned using the program MUSCLE v.3.8.31 (Edgar 2004), with manual adjustments made in Se-Al v.2.0a11 (Rambaut 2002). Gene clusters with large portions of poorly aligned positions and divergent regions (e.g., *trnL–trnF* [chloroplast *trnL* intron, *trnL* exon 2, and *trnL–trnF* intergenic spacer] and *GCYC* [Gesneriaceae *cycloidea* homolog]) were run through the Gblocks server (Castresana 2000; Talavera and Castresana 2007) to remove these regions. Gene trees were built for each cluster using maximum likelihood (ML) as implemented in the program RAxML v.7.2.8 (Stamatakis 2006). To assess uncertainty in the topologies, we performed 500 nonparametric bootstrap replicates using RAxML. Unambiguously spurious placements of taxa were manually checked and removed from these alignments. These removed sequences are not necessarily erroneous, but could instead represent alignment issues or sampling issues. We considered taxa to be "unambiguously spurious" when their placement in these analyses was clearly contradictory to their phylogenetic placement in the original publication of the data.

Outgroups were chosen from among Gentianales, Lamiales, and Solanales, based on inferred relationships of these groups to Gesneriaceae in recent phylogenetic studies (Schäfferhoff et al. 2010; Refulio-Rodriguez and Olmstead 2014). We gathered selected data from each of these studies to include at least one representative of each family in Lamiales. These studies were chosen based on broad sampling across Lamiales and included overlapping gene sampling with Gesneriaceae clusters. Individual gene clusters from each of these studies were first aligned in MUSCLE and then aligned to our own using the profile-to-profile command in MUSCLE. Gene trees and support values were assessed for the expanded clusters as described above.

DNA Extraction, Amplification, and Sequencing

For new sequences generated for this study, total genomic DNA was isolated from approximated 100 g of fresh leaf material using a modified CTAB protocol (Doyle and Doyle 1987). The nuclear ribosomal internal transcribed spacer (ITS) region and the chloroplast *trnL–trnF* were amplified by PCR (Roalson et al. 2003). The PCR protocol for nuclear and plastid markers consisted of a 25 μL sample containing 17.8 μL sterile dH_20, 2.5 uL 10× Thermopol reaction buffer with 20 mM Mg_2+ (New England Biolabs, Ipswich, MA), 1 μL 10 μM forward primer, 1 μL 10 μM reverse primer, 1.5 μL 2.5mM dNTP, 0.2 μL 5 U/μL Taq polymerase (New England Biolabs), and 1.0 μL diluted DNA template. The PCR conditions for the ITS region included initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 1 min, 48°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 5 min. The PCR conditions for the *trnL–trnF* marker included initial denaturation at 94°C for 1 min, followed by 34 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 1 min. All PCR products were visualized by 1% agarose gel electrophoresis and purified using ExoSAP-IT.

The 10 μL cycle sequencing reactions contained 6.67 μL sterile H_20, 0.33 μL 10 μM primer, 1.0 μL 5× sequencing buffer, 1.0 μL BigDye Terminator ver.3.1, and 1.0 μL cleaned PCR product. Cycle sequencing reactions included 25 cycles of 96°C for 10 s, 50°C for 5 s, with a final extension at 60°C for 4 min. Cycle sequencing products were purified using Performa DTR Gel Filtration Cartridges (EdgeBio), and DNA sequences were visualized on a 48-capillary 3730 DNA Analyzer (Applied Biosystems) at Washington State University. Contigs were assembled and edited using Sequencher.

Concatenation and Phylogenetic Analysis

After sequence alignments for each gene region were prepared, clusters were concatenated using Phyutility (Smith and Dunn 2008). We implemented the approach of Hinchliff and Roalson (2013) to improve the supermatrix alignment by scaffolding our matrix to the two most widely sampled markers. The nuclear ITS and plastid *trnL–trnF* intergenic spacer are the most widely sampled. Species without one or the other of these markers were eliminated from the final matrix. This approach has been shown to improve topology assessment and support (Hinchliff and Roalson 2013). Additionally, rogue taxa were assessed and pruned from 500 nonparametric bootstrap replicates generated with the full alignment using RogueNaRok v.1.0 (Aberer et al. 2013).

ML analyses were performed on the full supermatrix alignment using parser and ExaML v.3.0.2 (Kozlov et al. 2015). The starting tree for the ML search was obtained using a fast maximum parsimony heuristic implemented in RAxML. The ML analysis used the general time reversible model with gamma distributed rate heterogeneity. To assess uncertainty in the topology and branch-length estimates, we ran 500 nonparametric bootstrap replicates on the original data set. Bootstrap data sets were generated in RAxML (ML bootstraps) and summarized on the optimal ML topology using SumTrees v.3.3.1 (Sukumaran and Holder 2010). To investigate the effect of data partitioning, we ran ML searches for both a partitioned and unpartitioned data set. Data were partitioned by gene region.

Additional node support was calculated using the nonparametric Shimodaira–Hasegawa–Like (SHL) implementation of the approximate likelihood-ratio test (aLRT; Anisimova and Gascuel 2006). Estimation of aLRT values involves passing our best ML estimate of the phylogeny to RAxML, which does additional searches to produce a nearest-neighbor interchange (NNI)-optimized estimate of each branch in the tree. The aLRT statistic is calculated by comparing each branch in the best ML tree (NNI-optimized) with the second-best NNI configuration around the branch of interest (four adjacent branches). This optimization is needed to calculate the SHL version of the aLRT to estimate support values, which are then calculated by RAxML. We take a conservative view and consider SHL values of 85 or greater (i.e., a 15% or less chance that a branch is incorrect) as strong support (Guindon et al. 2010; Anisimova et al. 2011).

Divergence Times

Biogeographic reconstruction and ancestral character estimation methods require ultrametric trees. We dated the optimal ML tree and all bootstrap replicates to chronograms using penalized likelihood (PL), implemented in the program treePL (Smith and O'Meara 2012). Each bootstrap replicate was first estimated individually using the program ExaML to obtain branch lengths, followed by dating using treePL. Cross-validation of our calibration points tested seven values for the smoothing parameter (0.001, 0.01, 0.1, 1, 10, 100, and 1000) and resulted in an optimal smoothing parameter of 0.01. Confidence intervals for our dating estimates were produced from the bootstrap replicates using SumTrees.

Because there are no known fossils for Gesneriaceae, we relied on external fossil calibrations and geologic ages to estimate divergence times among lineages of Gesneriaceae. To calibrate the phylogeny, we identified 12 calibration points based on fossil and geologic data obtained from the literature. This approach of using a broad phylogeny to infer divergence times for an ingroup that lacks internal fossil calibration points has been shown to be successful in other studies for estimating divergence times (Janssens et al. 2009; Perret et al. 2013; Nazaire et al. 2014).

All fossil calibration points were chosen to provide minimum stem ages and included a distribution of node calibrations for outgroup nodes (Table 1). As all of the fossil calibration points reside outside of Gesneriaceae, we also applied maximum ages on stem or crown groups that are endemic to specific regions or islands. These included Lord Howe Island, the Hawaiian Islands, the Marquesas Islands, Fiji, and GAARlandia (Greater Antilles + Aves Ridge land bridge; Table 1). Finally, we placed conservative maximum ages at the crown of Lamiales and stem of *Coffea* as secondary calibration points based on estimates from Janssens et al. (2009).

Character State Coding

In the present study, seven features were scored: 1) primary corolla color (white, yellow, red, purple, green), 2) corolla shape (tubular, funnelform, salverform, campanulate, rotate, gibbous), 3) flower symmetry (actinomorphic, zygomorphic), 4) corolla gibbosity (non-saccate, saccate, spurred), 5) ovary position (superior, inferior), 6) epiphytism (yes, no), (7) growth form (caulescent, rosulate, unifoliate), and 8) pollination syndrome (generalist, melittophily [bee pollination], ornithophily [bird], euglossophily [euglossine bee], chiropterophily [bat], psychophily [butterfly], phalaenophily [moth], myophily [fly]). Pollination syndromes for bees (melittophily) and euglossine bees

TABLE 1. Calibration points used in divergence time estimation

Fossil	Minimum age	Clade assigned to	Topological position	Constraint minimum (myr)	Fossil reference	Used in:
Acanthus rugatus	Early–middle Oligocene	*Acanthus*	Stem	28.8	Reid and Chandler (1926)	Tripp and McDade (2014) (Acanthaceae); Nazaire et al. (2014) (*Mertensia*)
Ajuginucula smithii	Early–middle Oligocene	Lamiaceae	Stem	28.4	Reid and Chandler (1926)	Petrova et al (2013) (Scrophulariaceae)
Fraxinus wilcoxiana	Middle Eocene	*Fraxinus*	Stem	44.3	Call and Dilcher (1992)	Bell et al. (2010) (angiosperms); Smith et al. (2010) (angiosperms); Perret et al. (2013) (Gesneriaceae)
Paulownia inopinata	Middle Miocene	Paulowniaceae	Stem	16	Butzmann and Fischer (1997); Fischer and Butzmann (2006);Manchester et al. (2009)	Perret et al. (2013) (Gesneriaceae)
Cantisolanum daturoides	Middle Eocene	Solanales	Stem	44.3	Collinson et al. (1993)	Bell et al. (2010) (angiosperms); Smith et al. (2010) (angiosperms)
Unnamed (Bignoniaceae)	Early Eocene	Bignoniaceae	Stem	49.4	Wehr and Hopkins (1994); Pigg and Wehr (2002)	Bell et al. (2010) (Bignoniaceae); Perret et al. (2013) (Gesneriaceae)
Unnamed (Bignoniaceae)	Early Oligocene	*Catalpa*	Stem	35	Manchester (1999)	Bell et al. (2010) (Bignoniaceae)
Geological	**Maximum age**	**Clade assigned to**	**Topological position**	**Constraint maximum (myr)**	**Reference**	**Used in:**
Fiji	Late Eocene	Pacific *Cyrtandra*	Stem	40	Evenhuis and Bickel (2005)	Clark et al. (2008; 2009)
GAARlandia	Early Oligocene	Gloxinieae	Crown	35	Iturralde-Vinent and MacPhee (1999)	Roalson et al. (2008)
Hawaiian Islands	Early Pliocene	*Cyrtandra longifolia*	Crown	5.1	Price and Clague (2002)	Clark et al. (2008; 2009)
Lord Howe Island	Early Miocene	*Negria rhabdothamnoides*	Stem	23	McDougall et al. (1981); McDougall and Duncan (1988)	Woo et al. (2011)
Marquesas Islands	Late Miocene	*Cyrtandra feaniana*	Crown	6	Florence and Lorence (1997)	Clark et al. (2008; 2009)
Secondary	**Maximum age**	**Clade assigned to**	**Topological position**	**Constraint maximum (myr)**	**Reference**	
Lamiales	Middle Cretaceous	Lamiales	Crown	106.9	Janssens et al. (2009)	
Gentianales	Middle Cretaceous	*Coffea*	Stem	112.8	Janssens et al. (2009)	

(euglossophily) are considered separate as euglossine bees collect volatile compounds from the flowers as the reward rather than pollen or nectar. The states for these and other background information are provided (Supplementary Appendix 2, available on Dryad). States were scored from the literature. Uncertainties in the character state codings were treated as missing data in the analyses. Species that did not have clearly defined

character states were excluded from the appropriate analyses.

Primary corolla color can be variable within species; therefore, we scored character state based on our best judgment of what color constituted the majority of the corolla. For example, colors that appeared to be intermediate between yellow and red were divided based on their closer affinity to either color. Hence, yellow–orange was grouped into yellow and red–orange was grouped into red.

Additionally, corolla shapes were often described as intermediate shapes (e.g., tubular-funnelform). During these occurrences, character states were judged based on available images and drawings of the flowers. For example, in the case of tubular-funnelform flowers, these were scored as tubular if the basal portion of the corolla tube had a more-or-less parallel tube shape.

Pollination syndrome is a controversial topic in pollination biology because of possible limitations on the accuracy of predicting the true pollinator without first-hand observations (reviews in Fenster et al. 2004; Rosas-Guerrero et al. 2014; for exceptions see Smith S.D. et al. 2008, 2009 [*Iochroma*, Solanaceae] and counter arguments in Fenster et al. 2009). However, the predictability of pollination syndromes has been largely untested, with the exception of Armbruster et al. (2011), who found that pollination syndromes in *Dalechampia* are predictive among geographic areas, and Martén-Rodríguez et al. (2009) who found floral traits to be highly predictive of pollinators in Antillean Gesneriaceae. We think that they can be helpful in the study of floral patterns in the general sense of predicting the dominant pollinator. This is a position supported by the above referenced reviews of the literature, and we therefore use them here. We scored this character based on reports in the literature and our best judgment based on the other scored flower characters. Specifically, when reports were absent, a combination of primary flower color, corolla shape, and flower symmetry was used to score pollination syndrome. Ornithophilous (Aves) flowers were red, orange, or yellow, tubular, and zygomorphic. Psychophilous and Phalaenophilous (Lepidoptera) flowers were purple or blue, salverform, and zygomorphic. Euglossophilous (Euglossini) flowers were white or light purple, funnelform, and zygomorphic. Chiropterophilous (Chiroptera) flowers were white or green, campanulate, and zygomorphic. Myophilous (Diptera) flowers were white or pale purple, salverform, and zygomorphic. Melittophilous (Apidae) flowers were purple, white, yellow, or orange, campanulate or funnelform, and zygomorphic or actinomorphic. Scattered reports of the observed pollinators in gesneriads displaying some combination of floral characters were also used to score the possible pollinator (Snow and Teixeira 1982; Steiner 1985; Feinsinger et al. 1986; Stiles and Freeman 1993; Kastinger and Weber 2000; SanMartin-Gajardo and Sazima 2004; 2005a; 2005b; Gao et al. 2006; Martén-Rodríguez and Fenster 2008; Tang et al. 2009; Camargo et al. 2011; Bogacheva-Milkoteva et al. 2013; Guo and Wang 2014; Rodrigues and Rodrigues 2014). Potential pollinators in different geographic regions were considered if that pollinator was native or naturalized to a specific region where the gesneriad species occurred.

Geographic Areas

To reconstruct the timing of colonization and length of occupancy of the various pantropical regions, we used distribution information gathered from the World Checklist of Gesneriaceae (Skog and Boggan 2007) and the Global Biodiversity Information Facility (GBIF.org 2015). We assigned species to one or more regions in a global set of 11 biogeographic provinces. The 11 regions follow from commonly used definitions of regions, including Takhtajan's (1986) definitions of floristic provinces. Although some ambiguity about the limits of these regions exists, they correspond closely with geography and species distributions. We are defining these areas based on the distribution of Gesneriaceae species within those areas, not the full extent of the area (e.g., Europe obviously encompasses a broader area than listed, but the listed areas are the parts of Europe when Gesneriaceae are found). The areas are:

1. *Africa and Madagascar*—sub-Saharan Africa, South Africa, East Africa, Madagascar.
2. *Europe*—Pyrenees, Balkans, and Greece.
3. *South Asia*—India, Sri Lanka, Bangladesh, Nepal, Bhutan, western Myanmar.
4. *Southeast Asia and the Pacific*—southern China, Indochina, Indonesia, Oceania, Australia, New Zealand, Hawaii.
5. *East Asia*—Japan, Taiwan, eastern and central China.
6. *Temperate Andes*—Chile, Argentina.
7. *Tropical Andes*—northern Andes, Peru, Ecuador, Colombia, Bolivia, Venezuela.
8. *Amazon and the Guyana Highlands*—Amazon basin, Guyana.
9. *Atlantic Brazil*—Brazilian highlands, coastal Atlantic provinces of Brazil.
10. *Central America*—Panama north through Mexico.
11. *West Indies*—islands of the Caribbean.

Biogeographic Analyses

To test hypotheses of ancestral areas and broad-scale patterns of diversification in Gesneriaceae, we conducted ML-based analyses of historical biogeography. Several recently developed analytical

tools for historical biogeography exist, accounting for different historical biogeographic processes that might contribute to model fit, such as DEC and BayArea (Ree and Smith 2008; Landis et al. 2013). All of these models contain conceptually similar elements, and are now unified in the R package BioGeoBEARS (Matzke 2013a, 2013b). We initially explored five models that each account for different biogeographic processes (e.g., subset sympatry and vicariance). Each of these models was run either as unconstrained (i.e., all areas equally probable and no limitation on dispersal direction) or constrained (i.e., accounting for area connectivity and dispersal probabilities between regions). The five models tested initially were DEC, DEC+J, DIVALIKE, DIVALIKE+J, and BAYAREALIKE+J. The models with "+J" refer to those where founder events are allowed (Supplementary Appendix 3, available on Dryad). Because the BAYAREALIKE+J model only allows for exact range-copying sympatry, we wanted to explore if allowing subset sympatry would improve the fit of the model. This sixth model was the BAYAREALIKE+subset sympatry+J model (henceforth, "subset sympatry" abbreviated "s"). Subset sympatry was a fixed parameter in this model. Model fit was compared using LRT, AICc (Hurvich and Tsai 1989), and BIC criteria (Schwarz 1978).

One primary limitation in statistical biogeographic analysis of ancestral areas is not the size of the tree, but the number of areas. Because of current matrix-handling algorithms, our large-scale analyses were performed by reducing the number of total areas and the number of areas that can be occupied simultaneously by lineages. We took three approaches to address this issue. First, we conducted a global analysis of our tree using all Gesneriaceae lineages while reducing the number of areas to eight regions. We combined the temperate Andes with the tropical Andes, the Amazon with Brazil, and Central America with the West Indies. Secondly, we conducted an analysis on only the subfamily Gesnerioideae that is mainly concentrated in the New World. Taxa outside of this subfamily were pruned using Phyutility. For this analysis we defined seven regions, lumping 1–5 into a single region defined as the "Old World" and using 6–11. Thirdly, we conducted an analysis on the subfamily Didymocarpoideae that is concentrated in the Old World. Taxa outside of this subfamily were pruned using Phyutility. For this analysis we defined six regions, using areas 1–5 and combining areas 6–11 into a single region defined as the "New World."

We then reconstructed ancestral states for each of these approaches using the optimx routine in BioGeoBEARS for the six models. The fit of the models was compared with respect to the addition of the "J" parameter using the LRT to determine which provided a better fit to the data. These were run on the PL-dated phylogeny described above. Note that these estimations are the ancestral state probabilities under the globally optimum model, not the locally optimum estimation, or the single best estimate of joint history (Felsenstein 2004). These biogeographic models do not implement ways to account for taxa sampling; therefore, no attempt was made to correct for missing taxa.

Another consideration for biogeographic inference is the specification of area availability through time and connectivity matrices. Using well-established timelines for the emergence of these geographic regions allows for parameters such as dispersal multipliers, time-stratified dispersal, and area connectivity. We conducted additional analyses on each of the previous approaches while utilizing a temporal framework.

We chose to divide our model into three time slices that reflect important paleogeographical changes during the history of Gesneriaceae, between 80 and 50 Ma, between 50 and 35 Ma, and between 35 Ma and the present day. For each time slice, scaling factors for the dispersal rate between areas were scaled similar to Buerki et al. (2011). Contiguous areas were scaled to 1, non-contiguous regions within the same large area (NW or OW) were scaled to a factor of 0.5, and dispersal to an area not present during a time slice was scaled to 0. Area connectivity matrices were constructed in a binary format (0, absent; 1, present) for all regions during each time slice. The area connectivity matrices represent dispersals that could occur between areas while scaling matrices represent the probability of those dispersals.

During the period from 80 to 50 Ma, Gondwana landmasses were nearing their final separation and South America had separated from Antarctica (McLoughlin 2001). The traditional hypothesis was that major lineages of Gesneriaceae would have evolved in vicariance following the Cretaceous breakup of Gondwana (Burtt 1998). We wished to test this hypothesis by constraining dispersal to 0.5 between the NW and OW during this period. During the 50–35 Ma period in the OW, India had come into contact with Asia after separation from Madagascar (Beck et al. 1995; Storey 1995). Several gesneriad species are endemic to India and we wished to explore the ages of dispersal to the subcontinent. In the NW during this same period, both the uplifted tropical Andes and the West Indies were just beginning to form (Graham 2003; Garzione et al. 2008). Dispersal to these areas was constrained to 0.0001 and movement was allowed between the temperate Andes and Brazil. Geological evidence suggests that before 30 Ma only low hills occurred in the region today occupied by the northern and central Andes (Garzione et al. 2008) and that the West Indies were beginning to form (Iturralde-Vinent and MacPhee 1999). Therefore, dispersal was given a small probability of 0.0001 rather than 0 to allow potential movement into these regions. In the NW during the period from 35 Ma to the present, connections between South America and Central America were beginning to occur (Coates et al. 1992; Iturralde-Vinent and MacPhee 1999), the West Indies had formed (Iturralde-Vinent and MacPhee 1999; Graham 2003), and several periods of uplift in the tropical Andes were occurring (Gregory-Wodzicki 2000; Garzione et al. 2008). Area connections and dispersal between these regions were then constrained

to allow movement between contiguous regions. In the OW during this period, Southeast Asia had come into contact with Australia and the Pacific Islands were forming (Hall 1998; Neall and Trewick 2008). Areas connections and dispersal reflect these paleogeographical changes.

To test for the increased diversification rates in different geographic regions, we utilized the GeoSSE (Goldberg et al. 2011). GeoSSE extends the Binary State Speciation and Extinction Model (BiSSE) binary model to incorporate a third, polymorphic state for geographic characters, since taxa are often not endemic but present in more than one area/state (Goldberg et al. 2011). Biogeographic regions were defined the same as above, except that we excluded South Asia, Europe, and East Asia: occurrence of gesneriads in these regions is only marginal. The current version of GeoSSE accounts for random incomplete taxon sampling but only allows for the comparison of two areas, so we compared each biogeographic region against the pooled values in all other regions, that is, diversification and dispersal rates estimated for species distributed in the Andes region were compared with the values estimated for species in the remaining regions. For the selected regions we estimated ML parameters for a full GeoSSE model (seven parameters), where speciation, extinction, and dispersal rates are allowed to differ between areas. Additional constrained models were tested: same rates of within-region speciation ($\lambda A = \lambda B$, $\lambda AB = 0$, five parameters), of between-region speciation ($\lambda AB = 0$, six parameters), of dispersal between regions ($qA = qB$, six parameters), of within-region extinction ($\mu A = \mu B$, six parameters), Mk2 ($\lambda A = \lambda B$, $\lambda AB = 0$, $\mu A = \mu B$, four parameters), and Mk1 ($\lambda A = \lambda B$, $\lambda AB = 0$, $\mu A = \mu B$, $qA = qB$, three parameters). ML parameters were estimated across 100 randomly selected ultrametric trees. We accounted for random taxon sampling by utilizing the skeletal tree approach of Fitzjohn et al. (2009). Sampling schemes were based on our previous geographic state scoring to estimate the proportion of unsampled taxa in each region. Model fit was assessed using LRT, AICc, and BIC criteria. The parameters of the best-fit models were used as a prior for the Markov Chain Monte Carlo (MCMC) search. We parameterized the selected model with an exponential prior $1/(2r)$, where r was the character-independent diversification rate, as estimated from the GeoSSE–ML searches. This prior was used on the estimation of each parameter. The MCMC chain was run over 25 randomly selected ultrametric trees for 10,000 generations, and the first 10% were discarded as burn-in.

Ancestral Character Estimation

Stochastic character mapping, as implemented in the program SIMMAP v.1.5 (Bollback 2006), was used to sample histories from the posterior distribution for flower morphology, pollination syndromes, epiphytism, and growth form. As missing taxa cannot be included in SIMMAP analyses, no attempt was made to account for missing taxa. All taxa without clearly defined character states found in our literature search were pruned from all trees prior to analyses (e.g., if primary flower color was not defined for a taxon it was deleted from the analysis and pruned from the trees). SIMMAP uses a stochastic algorithm to map discrete character states onto a distribution of phylogenetic trees and then summarizes character history statistics across all individual mappings. Therefore, this method incorporates topological and branch-length uncertainty contained in the distribution of trees (Bollback 2006). We used the dated bootstrap trees generated previously as the tree distributions for stochastic mapping in SIMMAP. We generated 10 character history mappings for each tree, ultimately providing 50,000 character histories for each trait. The bias parameter used was an empirical prior and the rate parameter was a gamma distribution prior using values of $\alpha = 1.25$ and $\beta = 0.25$. Rates of change among character states were averaged across all estimations to yield a mean per-tree number of transitions for each possible combination of character states in the analysis.

Diversification Analyses

BAMM was used for speciation–extinction analyses on the phylogeny (Rabosky et al. 2014; Rabosky et al. 2014a). This method allows detection and quantification of heterogeneity in evolutionary rates using reversible jump MCMC. To account for non-random incomplete taxon sampling in our analysis, we specified the sampling fraction for each tribe or subtribe in the family (the taxonomy and species numbers are given in Supplementary Appendix 2, available on Dryad). Chains were run for 50 million generations and sampled every 10,000 generations. After plotting the likelihoods of the sampled generations, the first 10% were discarded as burn-in and the effective sample size for likelihood and number of shifts was calculated to assess convergence. Event data generated from BAMM was then analyzed using the R package BAMMtools (Rabosky et al. 2014b). The location of significant rate shifts was inferred by sampling from all possible sets of shift configurations, and noting the nodes where the posterior probabilities summed to 0.95. Lineage-through-time plots were generated using the R packages "phytools" (Revell et al. 2012) and "laser" (Rabosky 2006).

In order to test for broad-scale character evolutionary patterns, we tested discrete models for corolla shape, color, gibbosity, and epiphytism using the R package "geiger" (Harmon et al. 2008). Model testing was implemented across the entire tree as well as subtrees for the Gesnerioideae and Didymocarpoideae subfamilies. Model testing does not account for missing taxa; therefore, no attempt was made to account for missing taxa. Pagel's delta, kappa, and lambda were used to test for signals of adaptive radiation, punctuational evolution, and phylogenetic covariance on the trees, respectively (Pagel 1999). The significance of each

parameter estimation over null models of no signal was assessed using the LRT.

The BiSSE (Maddison et al. 2007), as implemented in the "diversitree" package (v. 0.9-1, Fitzjohn 2012), was used to estimate speciation, extinction, and transition rates for ornithophily, epiphytism, and growth form. We analyzed the ornithophilous syndrome against all others (including melittophily, psychophily, euglossophily, myophily, and chiropterophily) to understand the potential role of birds in the diversification of gesneriads. Epiphytism is widespread in the species-rich subtribe Columneinae. We chose to test the role of the epiphytic growth habit in diversification. Finally, the species-rich African endemic genus *Streptocarpus* exhibits diverse and unique growth forms including the presence of many unifoliate species.

Each of the three binary traits (ornithophily, epiphytism, and growth form) was analyzed on the entire tree and separately on the subfamilies Gesnerioideae and Didymocarpoideae. We tested eight models, including the full model where all six parameters are estimated and constrained models where we tested the effects of the trait on specific parameters. The models were: 1) full BiSSE model, six parameters; 2) equal speciation ($\lambda 0 = \lambda 1$), five parameters; 3) equal extinction rates ($\mu 0 = \mu 1$), five parameters; 4) equal transition rates ($q01 = q10$), five parameters; 5) no reversals ($q10 = 0$), five parameters; 6) Mk2 model ($\lambda 0 = \lambda 1$, $\mu 0 = \mu 1$), four parameters; 7) Mk1 model ($\lambda 0 = \lambda 1$, $\mu 0 = \mu 1$, $q01 = q10$), three parameters; and 8) Mk2 and no reversals ($\lambda 0 = \lambda 1$, $\mu 0 = \mu 14$, $q10 = 0$), three parameters. ML estimates for each model were conducted across 100 randomly selected ultrametric trees to account for phylogenetic uncertainty. We accounted for random taxon sampling by utilizing the skeletal tree approach of Fitzjohn et al. (2009). Sampling schemes were based on the proportion of coded states for each character present in the sampled taxa. For example, if the coded characters were 40% ornithophilous and 60% non-ornithophilous, we used values of 0.4 and 0.6 for the incomplete sampling scheme. Models were compared using the LRT, AICc, and BIC criteria. The best-fit model was then used to estimate parameters using the MCMC approach implemented in "diversitree." We parameterized the selected model with an exponential prior $1/(2r)$, where r was the character independent diversification rate, as estimated from the BiSSE–ML searches. This prior was used in the estimation of each parameter. The chain was run across 25 randomly selected ultrametric trees for 10,000 generations and the first 10% were discarded as burn-in.

Results

Taxon Sampling and Phylogenetic Analyses

Orthologous gene clusters were downloaded from PhyLoTa for 26 gene regions, with initial taxa sampling ranging from 18 (*26S*) to 945 (*trnL–trnF*; Supplementary Appendix 4, available on Dryad). Gblocks was used to remove positions of poor alignment and high divergence in three gene clusters: *atpB–rbcL*, *GCYC*, and *trnL–trnF*. Gblocks removed 40.5%, 57.4%, and 80.6% of the sequence alignment in each of these gene regions, respectively (Supplementary Appendix 4, available on Dryad). Removal of spurious taxa based on gene tree construction ranged from 0 to 102 taxa (in *trnL–trnF*; Supplementary Appendix 4, available on Dryad). New sequences were generated for ITS and *trnL–trnF* in *Achimenes warszewiciziana* (GenBank Accessions KT945236 [ITS], KT945237 [*trnL–F*]).

All gene clusters were subsequently aligned into a supermatrix containing 1033 species. There were 163 species that had neither ITS nor *trnL–trnF* sequences. These were removed resulting in 870 remaining species. RogueNaRok identified 44 rogue taxa whose removal improved the relative bipartition information criterion (RBIC) from 55.5% to 60.0%. The RBIC is the sum of all support values in the tree pruned of rogue taxa divided by the maximum possible support in a fully bifurcating tree with the initial set of taxa. The 44 rogue taxa were removed producing a final alignment of 826 species.

Our phylogenetic analyses were conducted on a sampling of 768 Gesneriaceae species and 58 outgroups for a total species sampling of 826 taxa. The concatenated matrix included 26 gene regions and 29,143 aligned base positions (Supplementary Appendix 4, available on Dryad; TreeBASE submission 18407). As with most mega-phylogeny analytical approaches, a significant proportion of the aligned cells are missing data (including indel-associated gaps), and 91.51% of the analyzed data set here was missing. Despite this high percentage of missing data, our phylogenetic analyses resulted in robust phylogenetic hypotheses that largely agree with previously published phylogenies (Supplementary Fig. S1, available on Dryad). Given space constraints and the fact that the taxonomic implications of the phylogeny are ancillary to the primary focus of this article, discussions of the taxonomic results are presented in Supplementary Appendix 5, available on Dryad.

Divergence Times

Divergence times were estimated on the ML tree and across the bootstrap set using treePL (Supplementary Fig. S2, available on Dryad), with multiple calibration points (Table 1), and the means, minimums, and maximums for stems and crowns of interest estimated (Fig. 1; Supplementary Appendix 6, available on Dryad). Most age estimates fall within the range found in previous estimates of clade ages for Gesneriaceae (Roalson et al. 2008; Bell et al. 2010; Woo et al. 2011; Perret et al. 2013; Petrova et al. 2015). Some differed to a degree (core Gesneriaceae crown, 69.60 (48.20, 77.06) this study; 44.7 (37.1, 60.5) Perret et al. 2013). When they differ, the ages estimated here are generally older than previous estimates. This could be due, at least in part, to the much denser sampling of the entire Gesneriaceae

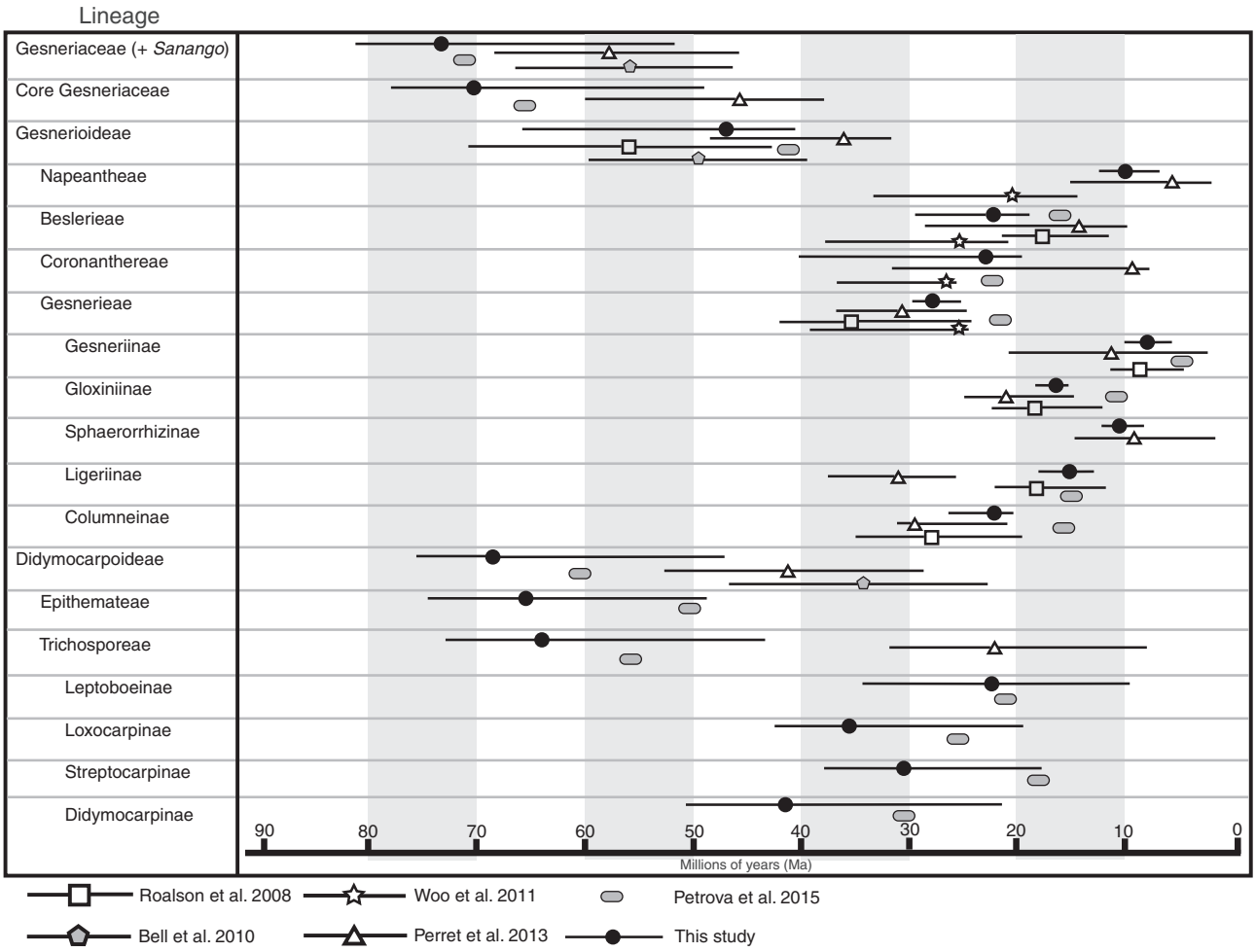

FIGURE 1. Bar chart indicating Gesneriaceae crown clade ages comparing the estimates from this study to previously proposed age estimates. Symbols represent the mean age estimate and bars represent the minimum to maximum range of the estimate. Numerical presentation of means, max and min ages, and methods used for each study are presented in Supplementary Appendix 6, available on Dryad.

here than previous studies (391 Didymocarpoid species in this study; 3 in Perret et al. [2013]), and the more even tribe/subtribe sampling of Gesnerioideae here than previously (we sample 72 Ligeriinae in our sampling of 376 Gesnerioideae [19%]; Perret et al. [2013] sampled 78 Ligeriinae of 199 Gesnerioideae [39%]). As Ligeriinae only represent approximately 6% (91 of 1200+ species) of Gesnerioideae diversity, these sampling effects could explain some of the different age estimates. Further, previous studies such as Perret et al. (2013) did not assess their data for rogue taxa and these could also have an effect on branch-length estimates used for ultrametricizing trees if some branch lengths were spurious.

Biogeographic Analyses

Six unconstrained and six constrained models were compared for model fit in BioGeoBEARS, and the best-fit model for both constrained and unconstrained sets was the BAYAREALIKE+s+J, with the constrained version of this model the best overall (Supplementary Appendix 3, available on Dryad). Given the limitations of area number on a phylogeny this large, the historical biogeographic reconstruction under this model provides general broad-scale patterns (Supplementary Fig. S3, available on Dryad). As has been previously found, Gesneriaceae originated in Andean (western) South America, with a dispersal to eastern Asia/Southeast Asia at approximately 70 Ma (Fig. 1; Supplementary Fig. S3; and Supplementary Appendix 6, available on Dryad), establishing the Didymocarpoideae lineage. This lineage persisted and predominantly diversified in Asia, with dispersals to Europe (Ramondinae clade; Fig. 1; Supplementary Appendix 6, available on Dryad) at around 40 Ma and Africa (Streptocarpinae clade; Fig. 1; Supplementary Appendix 6, available on Dryad) by around 35 Ma. Invasion of the Pacific islands occurred at approximately 11 Ma (*Cyrtandra*; Supplementary Appendix 6, available on Dryad). There were a number of inferred dispersal or subset sympatry splits among East Asia, Southeast Asia, and the Indian subcontinent, but a large proportion of the deeper nodes appear

to be concentrated in Southeast Asia (Supplementary Fig. S3, available on Dryad). Finer-scale patterns can be inferred from the separate reconstructions on the NW and OW clades because they have larger numbers of areas that were not clumped together as in the family-wide reconstruction (Supplementary Figs. S4 and S5, available on Dryad). This is particularly true of the NW tree, where the origin is placed in the temperate Andes with movement north and east into the tropical Andes and Brazil.

In the New World, the most significant dispersal events were from the Andes to Amazonia/Atlantic Brazil at around 27 Ma (Gesnerieae clade; Fig. 1; Supplementary Appendix 6, available on Dryad), and to the South Pacific (Coronanthereae clade; Fig. 1; Supplementary Appendix 6, available on Dryad) at around 23 Ma. The Amazonia/Brazilian lineage dispersed north to the Caribbean and Central America by 20 Ma (Gesneriinae/Gloxiniinae clade; Fig. 1; Supplementary Appendix 6, available on Dryad), and back to Andean South America by 22 Ma (Columneinae clade; Fig. 1; Supplementary Appendix 6, available on Dryad).

GeoSSE analyses compared diversification rate statistics and model fit between individual geographic areas and all other areas combined, for a total of five comparisons (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7, available on Dryad). Although these comparisons are by their nature not independent, comparing the overlapping patterns can provide information as to the processes important to diversification in different areas in relation to a general background rate. When Africa and Madagascar are compared with all other areas combined, the "equal q between regions" six-rate model or Mk2 (4-rate) model is selected as the best fit depending on the statistic used (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7, available on Dryad). Temperate and Tropical Andes best fit the "equal λ within-regions" five-rate model, while Amazon and Atlantic Brazil best fits the "no λ between-regions" six-rate model (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7, available on Dryad). Central America and West Indies best fit is either the "equal λ within-regions" five-rate model or "equal μ within-regions" six-rate model, depending on the statistic used, and the Pacific and Southeast Asia best fit is the "no λ between-regions" six-rate model (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7, available on Dryad). For each of the area comparisons, the estimated model parameters are as important as the model chosen. For instance, for Africa and Madagascar, the best-fit model suggests a lower speciation rate for species endemic to the area than averaged across other areas (with the six-rate model), or equal speciation and extinction (Mk2 model; Supplementary Appendix 7A, available on Dryad). Similarly, the best-fit model for Temperate and Tropical Andes has equal speciation rates for lineages endemic to the Andes as those outside the Andes, but the extinction rate in the Andes is substantially lower than outside the Andes, possibly supporting the "museum hypothesis" of some authors (Stebbins 1974). None of the best-fit models for an area support that focal area as having significantly higher speciation rates within the area than the rest of the areas combined, with the possible exception of Central America and the West Indies, where one of the two possible best-fit models estimates a higher speciation rate for the area than background (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7D, available on Dryad). New World regions do show higher net diversification rates overall, although the contributing factors are different in different areas (such as the lower extinction rate in the Andes; Fig. 2; Supplementary Appendix 7, available on Dryad).

Ancestral Character Estimations

Characterization of directionality and frequency of floral shape, color, and overall inferred pollination syndrome definitely supports a dynamic pattern of floral morphological change across Gesneriaceae (Supplementary Fig. S7; and Supplementary Appendix 8, available on Dryad). It is clear that there are marked differences in the patterns found in the New World Gesnerioideae clade and the Old World Didymocarpoideae clade. These differences are particularly stark when primary flower color and inferred pollination syndrome are compared (Supplementary Appendix 8, available on Dryad). Although Gesneriaceae as a whole have similarly large numbers of shifts between mellitophily/generalist and ornithophily (mellit. to ornith. average number of transitions 163.47, ornith. to mellit. 197.50), when the two major subtrees are compared separately, there are clearly different underlying patterns. Gesnerioideae is dominated by shifts between mellitophily and ornithophily, but Didymocarpoideae has more balance among all of the inferred pollination syndromes. Pollination syndrome is obviously a compound character, made up of several interacting characteristics. The two components that have a large effect on pollination syndrome are flower shape and primary flower color (Martén-Rodríguez et al. 2009; Fernández-Mazuecos et al. 2013; Gómez et al. 2014). Although pollination syndrome patterns differ between the Old and New World lineages, this is not true of both of these underlying traits (Supplementary Appendix 8, available on Dryad). Flower color patterns are distinctive in the two clades (Supplementary Fig. S7, available on Dryad); the Gesnerioideae transition matrix is dominated by transitions from all of the other colors to red (Supplementary Appendix 8, available on Dryad), while the Didymocarpoideae are dominated by transitions (in both directions) between white and purple (Supplementary Fig. S7; and Supplementary Appendix 8, available on Dryad). Conversely, flower shape dynamics between Gesnerioideae and Didymocarpoideae are quite similar, with similar

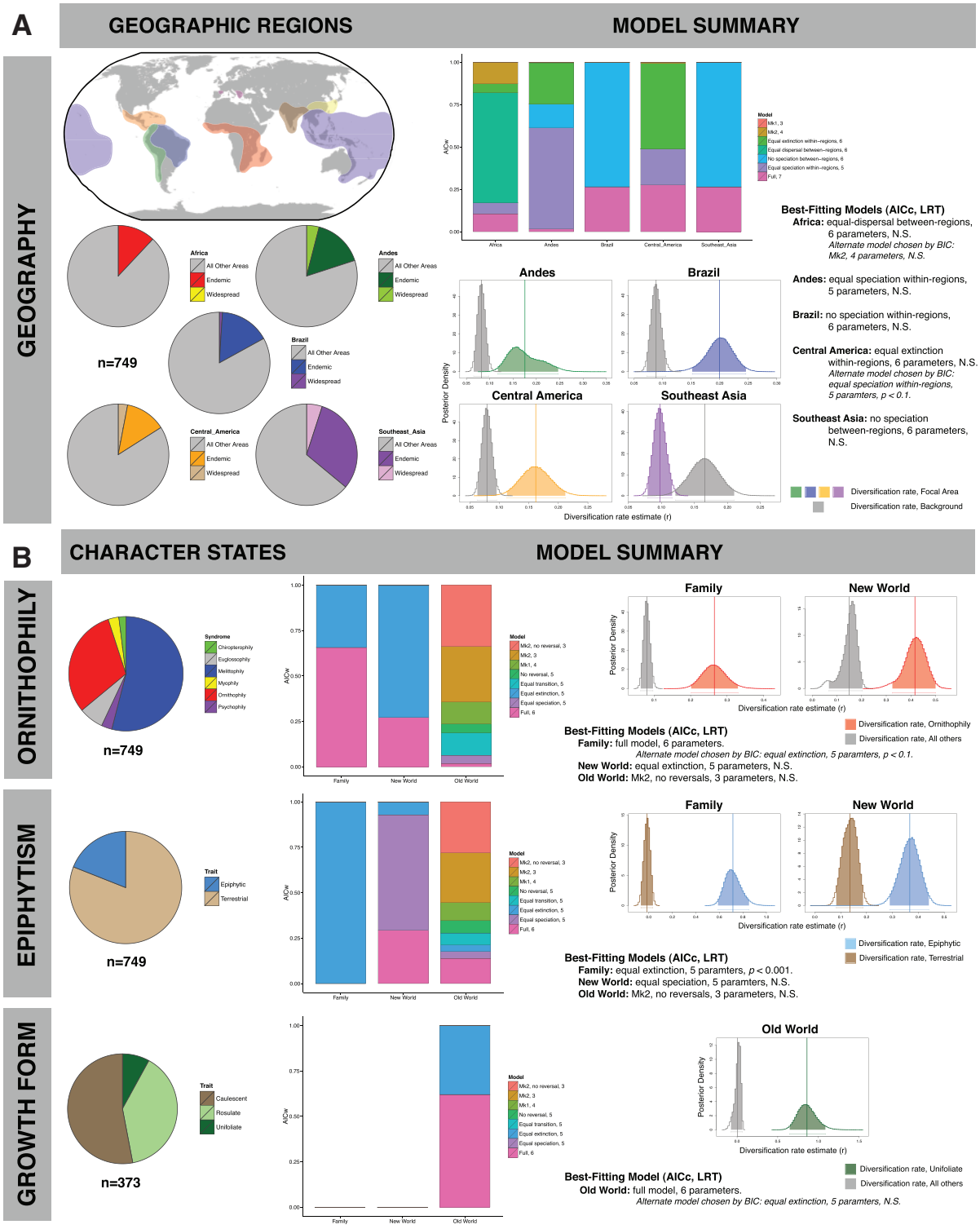

FIGURE 2. Summary of geography and character diversification dynamics from the GeoSSE and BiSSE analyses. a) Geographic areas, sampling distribution for each area, cumulative AICw for areas by model, and diversification rate estimates. Geographic areas are colored on the map and pie charts as follows: orange, Central America; green, Andes; blue, Brazil; red, Africa; pink, Europe; brown, South Asia; yellow, East Asia; purple, Southeast Asia. Full GeoSSE model details and results are presented in Supplementary Fig. S6 and Supplementary Appendix 7, available on Dryad. b) Character state distributions for ornithophily, epiphytism, and growth form, AICw for each character compared by family and separate subfamilies, and best-fitting models for each character. Full BiSSE model details and results are presented in Supplementary Figs. S8 and S10 and Supplementary Appendix 11, available on Dryad. Abbreviations: AICw, Akaike Information Criterion weights.

distributions among the different shape categories, and dominance in transitions between tubular and funnelform, and to a lesser degree campanulate (Supplementary Appendix 8, available on Dryad). Character state transitions in vegetative characters are very asymmetrical, with a preponderance of shifts from epiphytic to non-epiphytic habit and from unifoliate to non-unifoliate (Supplementary Appendix 9, available on Dryad).

Diversification Analyses: BAMM, Geiger, and BiSSE

The analyses of rate variation across Gesneriaceae strongly support several significant changes in diversification rate in both major clades (Fig. 3; Table 2). These correspond to Pacific *Cyrtandra* (Fig. 3d), core *Streptocarpus* (Fig. 3e), core Columneinae (Fig. 3f), core *Nematanthus* (Fig. 3g), and Beslerieae (Fig. 3h). When the macroevolutionary rate regimes are compared among clades of the tree (Fig. 4), several overlapping patterns are found. First, the branches of the earliest lineage splits of both major clades share a similar rate (S1; Fig. 4). Although it appears that the background Gesnerioideae stem is much longer than the background Didymocarpoideae stem, this shared rate appears to be due to the sparsely branched nature of the grade of lineages leading to the core of the two subfamily clades (inferred slow diversification rate, including the long core Gesnerioideae stem (Fig. 4, dark blue branches). Most of the lineages within the two main clades clearly share a more similar rate than with any other lineages (background Gesnerioideae [S3] and background Didymocarpoideae [S6] rates; Fig. 4). Within Gesnerioideae, the rates found in Beslerieae, core Columneinae, and core *Nematanthus* are all distinct from the background core Gesnerioideae rate (Fig. 4). Similarly, core *Streptocarpus* and Pacific *Cyrtandra* both have macroevolutionary rate regimes distinct from the background core Didymocarpoideae rate and each other. Similar results have been found in some other groups where this has been studied (McGuire et al. 2014; Rabosky et al. 2014a), but these studies have generally not found so many distinct rate classes in one lineage.

Geiger analyses provide insights into the rate characteristics of characters of interest, particularly the phylogenetic signal of those characters (Pagel's lamda), and trait evolution at speciation (Pagel's kappa). These tests were made for Gesneriaceae as a whole and the Gesnerioideae and Didymocarpoideae subtrees separately (Supplementary Appendix 10, available on Dryad). Primary flower color best fit the "all-rates-different" (ARD) model for Gesneriaceae, ARD for Gesnerioideae, and ARD for Didymocarpoideae. Corolla shape best fit ARD for Gesneriaceae, ARD for Gesnerioideae, and the "symmetric" model (SYM) for Didymocarpoideae. Corolla gibbosity best fit ARD for Gesneriaceae, SYM for Gesnerioideae, and ARD for Didymocarpoideae. Epiphytism best fit ARD for Gesneriaceae, ARD for Gesnerioideae, and SYM for Didymocarpoideae. When considered as a whole, Gesneriaceae demonstrate significant phylogenetic signal for flower color, flower shape, and epiphytism, and significant association of trait evolution with speciation for the same characters. When the two subtrees are considered separately, flower gibbosity is also significant for Gesnerioideae for both statistics, and epiphytism is not significant for either measure in Didymocarpoideae. These results support a punctuational model of evolution (character change at speciation events) for flower shape and flower color across Gesneriaceae, and possibly for floral gibbosity and epiphytism in Gesnerioideae.

BiSSE analyses on the influence of ornithophily on diversification rates demonstrate clear differences in the importance of ornithophily between Gesnerioideae and Didymocarpoideae (Fig. 2; Supplementary Fig. S8; and Supplementary Appendix 11D–F, available on Dryad). When ornithophily is fitted to the whole Gesneriaceae phylogeny, the full six-rate model or the five-rate equal μ model is best, depending on the statistic used (Fig. 2; Supplementary Fig. S8; and Supplementary Appendix 11D, available on Dryad). When the New World Gesnerioideae tree is used, the five-rate equal μ model is best (Supplementary Appendix 11E, available on Dryad), and for the Didymocarpoideae subtree, the equal λ, equal μ, $q1=0$ three-rate model is best (Supplementary Appendix 11F, available on Dryad). This more significant influence of ornithophily on the Gesnerioideae clade is also noticeable from the differences in SIMMAP ancestral character estimates of primary flower color on the two subtrees, since red/orange/yellow colors are associated with bird pollination (Supplementary Fig. S7, available on Dryad). Whether tested on the full tree or the New World subtree, ornithophily is modeled as having a large impact on speciation rate.

For the influence of epiphytism on diversification rates, BiSSE analyses provide different results depending on whether the whole tree or the two subfamily-level subtrees are used to test for correlated changes (Fig. 2; Supplementary Fig. S8; and Supplementary Appendix 11A–C, available on Dryad). When the entire tree is considered, the BiSSE best-fit model is the five-rate model with equal extinction rates (μ; Supplementary Appendix 11A, available on Dryad). However, when the inferred pattern of change in this character is visualized on the trees (via SIMMAP, Supplementary Fig. S9, available on Dryad), it is clear that epiphytism is much more of a factor in diversification of New World Gesnerioideae, particularly the core Columneinae and core *Nematanthus* clades, both of which BAMM infers to have elevated diversification rates. When epiphytism is analyzed with BiSSE separately for Gesnerioideae and Didymocarpoideae (Supplementary Appendix 11B–C, respectively, available on Dryad), it is clear that these clades have very different model fit. The didymocarpoid clade fits a three-rate model (equal λ, equal μ; $q1=0$), while the gesnerioid best fit is the five-rate equal speciation rate model (Supplementary Fig. S8; and Supplementary Appendix 11B, available on Dryad). This

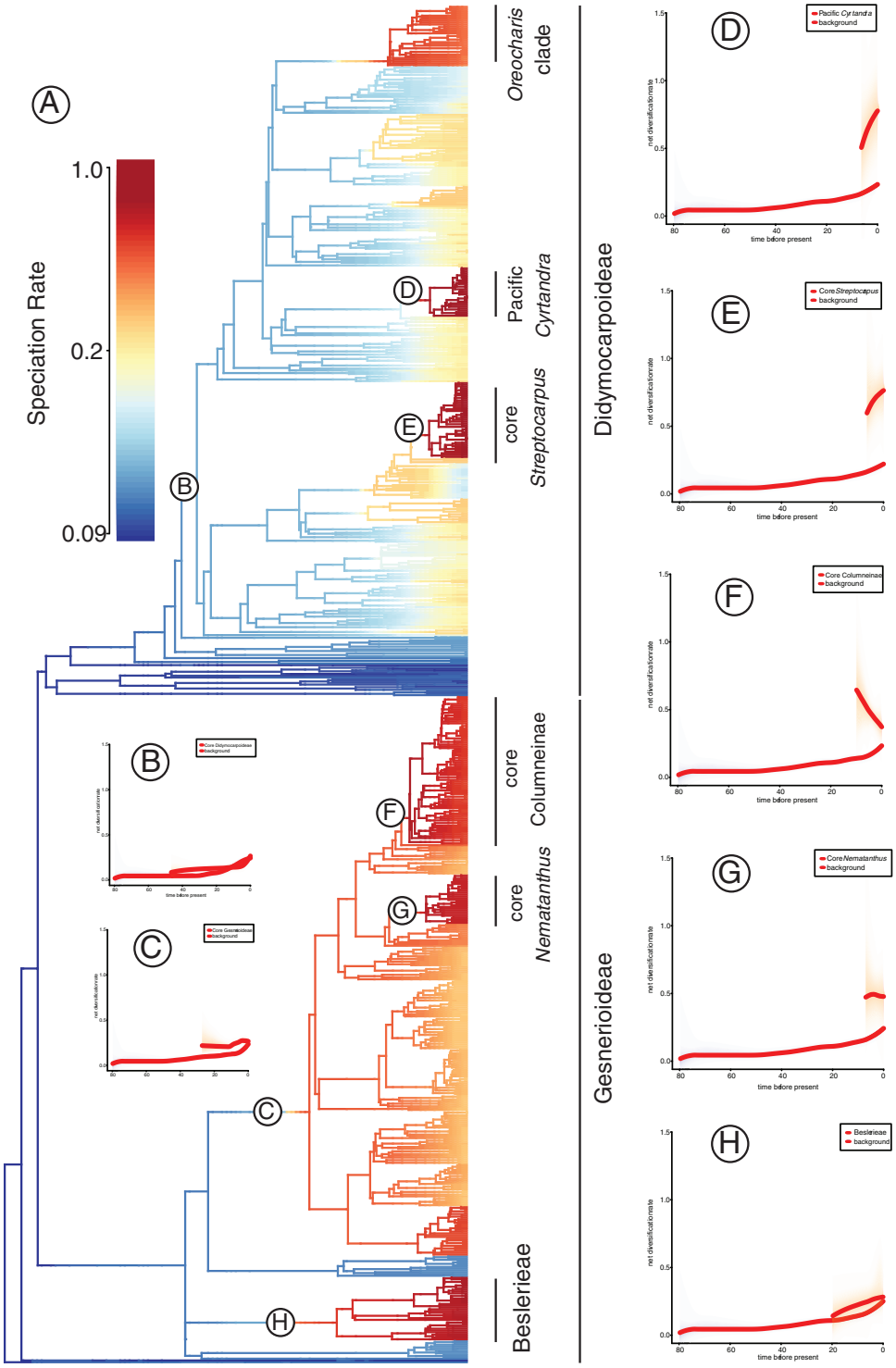

FIGURE 3. Mean phylorate plot for speciation using the time-calibrated ML phylogeny and net diversification rate plots for focal clades. a) Mean phylorate plot with colors along branches to denote the instantaneous rate of speciation at each time point. The mean phylorate reflects the mean of the marginal posterior density of speciation rates on a localized segment of the phylogenetic tree. See Table 2 for numerical rate estimates. Clades with rate shifts discussed in the text are named. b) Core Didymocarpoideae net diversification rate plot compared with the background diversification rate. c) Core Gesnerioideae net diversification rate plot compared with the background diversification rate. d) Pacific *Cyrtandra* net diversification rate plot compared with the background diversification rate. e) Core *Streptocarpus* net diversification rate plot compared with the background diversification rate. f) Core Columneinae net diversification rate plot compared with the background diversification rate. g) Core *Nematanthus* net diversification rate plot compared with the background diversification rate. h) Beslerieae net diversification rate plot compared with the background diversification rate.

TABLE 2. Overall diversification regime and diversification rate shifts, as inferred from BAMM analyses

Name	Taxon	Speciation rate (λ)	Extinction rate (μ)	Diversification rate
Overall	Gesneriaceae	0.2266956 [0.205, 0.254]	0.0648856 [0.038, 0.010]	0.20207
Core Gesnerioideae	Gesnerioideae	0.2956305 [0.268, 0.329	0.0408366 [0.013, 0.084	0.25479
Core Gesnerioideae background		0.2041495 [0.177, 0.238]	0.0727512 [0.389, 0.116]	0.13140
Beslerieae	*Anetanthus*, *Besleria*, *Cremosperma* *Gasteranthus*, *Tylopsacas*	0.6466491 [0.412, 0.945	0.3916895 [0.096, 0.738]	0.25496
Beslerieae background		0.2183877 [0.198, 0.244]	0.0584205 [0.032, 0.093]	0.15997
Core *Nematanthus*	*Nematanthus*	0.5850695 [0.431, 0.776	0.1134831 [0.008, 0.327]	0.47159
Core *Nematanthus* background		0.2217864 [0.200, 0.249]	0.0642199 [0.037, 0.010]	0.15757
Core Columneinae	*Alloplectus*, *Columnea*, *Drymonia*, *Glossoloma*, *Neomortonia*	0.5060036 [0.425, 0.607	0.0686941 [0.004, 0.195	0.43731
Core Columneinae background		0.2137454 [0.192, 0.241]	0.0647090 [0.037, 0.101]	0.14904
Core *Streptocarpus*	*Streptocarpus*	0.8810439 [0.699, 1.121]	0.1599751 [0.010, 0.460]	0.72107
Core *Streptocarpus* background		0.2172357 [0.198, 0.244]	0.0635109 [0.036, 0.098]	0.15372
Pacific *Cyrtandra*	*Cyrtandra*	1.1297460 [0.776, 1.628]	0.4529740 [0.035, 1.088]	0.67677
Pacific *Cyrtandra* background		0.2191003 [0.198, 0.246]	0.0616215 [0.035, 0.097]	0.15748
Core Didymocarpoideae	Didymocarpoideae	0.2151116 [0.186, 0.255]	0.0553018 [0.017, 0.110]	0.15981
Core Didymocarpoideae background		0.2390638 [0.210, 0.275]	0.0751182 [0.040, 0.118]	0.16395

is notable because it suggests that the epiphytes have the same speciation rate as the non-epiphytes, but have a much lower extinction rate.

When diversification rates are modeled comparing unifoliate plants to other growth forms, the best-model fit is either the full six-rate model or the five-rate equal μ model, depending on the statistic used (Supplementary Fig. S8; and Supplementary Appendix 11G, available on Dryad). Under the six-rate model, unifoliate species have a much higher speciation rate than non-unifoliate species ($\lambda = 1.7239$ vs. $\lambda = 0.0897$), and also have a significantly higher extinction rate ($\mu = 1.2996$ vs. $\mu = 0.0531$). Under the five-rate equal μ model, unifoliate species are modeled to have significantly higher speciation rates ($\lambda = 0.9175$ vs. $\lambda = 0.0879$), albeit lower estimated rates than when extinction is modeled with two rates as above. It should be noted that only approximately 9% of the Didymocarpoideae diversity is unifoliate—less than the suggested 10% threshold for reasonable power for this test. The inferred significant effect of unifoliate growth on diversification rates therefore needs to be viewed with some caution (Davis et al. 2013).

DISCUSSION

It is clear from the analyses presented here that there are complex interactions among geography, floral form, and growth form in shaping the diversification patterns and rates of Gesneriaceae. The diversification rate shifts modeled here (Figs. 3–4) cannot be attributed to single factors such as geography, floral form, or growth form, nor to the variability of one of these characteristics. Instead, it appears that the significant diversification rate shifts and differences in rate shifts among clades are attributable to different sets of interacting forces.

The influence of geography on diversification patterns and rates is well established, at least in some cases (e.g., island biogeography, Carlquist 1974; Wagner and Funk 1995; Price and Wagner 2004; latitudinal diversity gradient, Jansson and Davies 2008; New World vs. Old World tropical plant diversity, Antonelli and Sanmartín 2011; Antonelli et al. 2015; see also "dispersification" sensu Moore and Donoghue 2007). Here, we explore this question primarily from the perspective of the impact of lineage residence in a particular geographic area on diversification rate. When the influence of geographic area on diversification is modeled (Fig. 2; Supplementary Fig. S6; and Supplementary Appendix 7, available on Dryad), different models fit the different focal area versus background comparisons, but none of the areas are suggested to have significantly higher speciation rates for the focal area under the best model(s) than the background of all other areas. This would suggest that being in a particular area is not enough to increase diversification rates. This is counter to what has been found for some other lineages for some of these areas (and other areas), such as the influence of dispersal into the Andes by *Lupinus* (Drummond et al. 2012), diversification of Aizoaceae and *Pelargonium* (among others) in southern Africa (Valente et al. 2014; Jones et al. 2013; Martínez-Cabrera and Peres-Neto 2013), and movement of Dipsacales lineages into different mountainous areas (Moore and Donoghue 2007). Some of the lineages which are supported as having elevated diversification rates (core Columneinae, core *Streptocarpus*, and Pacific *Cyrtandra*; Fig. 3; Table 2) are largely or wholly restricted to geographic areas we delimit in our analyses, but apparently when combined with other lineages in these areas there is not a demonstrable area effect on diversification rates. Previous study of diversification rates across angiosperms has suggested that there are differences in speciation and extinction rates between the New World and Old World (Antonelli et al. 2015); however, that overall pattern is not found in Gesneriaceae (Figs. 1 and 3; Supplementary Appendix 6, available on Dryad). Although the two subfamilies that are each almost

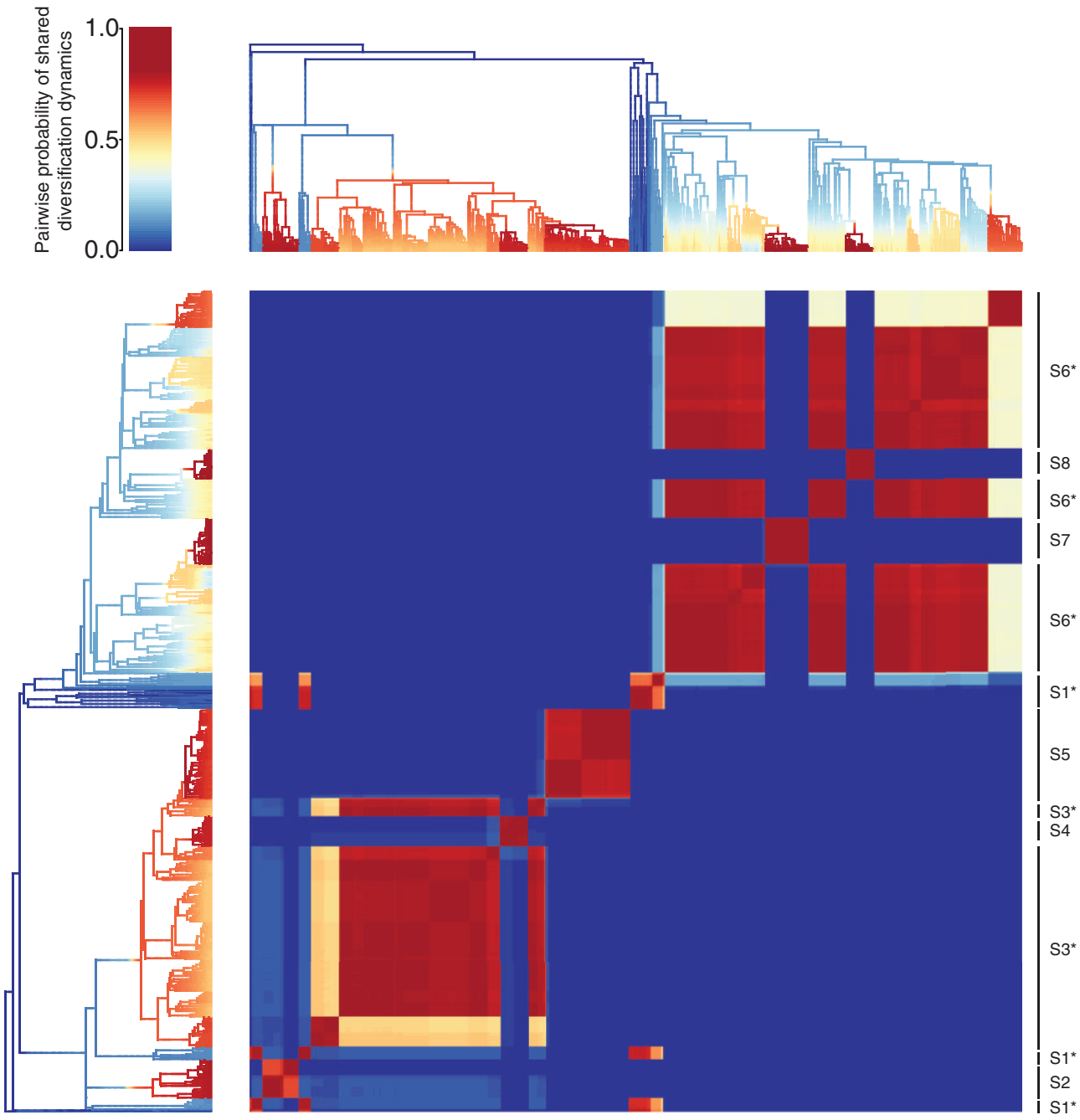

FIGURE 4. Speciation cohort matrices for Gesneriaceae. Each cell of the matrix is coded by color for the pairwise probability that two species share a common macroevolutionary rate regime. The mean phylorate plot trees are shown above and to the left of the cohort matrix for reference. Major cohorts of taxa that share particular rate dynamics are labeled to the right of the plot. The major cohorts are: S1, background Gesneriaceae; S2, Beslerieae; S3, background Gesnerioideae; S4, core *Nematanthus*; S5, core Columneinae; S6, background Didymocarpoideae; S7, core *Streptocarpus*; and S8, Pacific *Cyrtandra*.

exclusively either New World or Old World have different diversification rate patterns, both demonstrate lineages with high speciation rates, high extinction rates, and high and low overall diversification rates (Figs. 1 and 3; Supplementary Appendix 6, available on Dryad).

There is support for some clades fitting the "museum hypothesis" of diversity accumulation (Stebbins 1974). For instance, the Andes region is modeled to have the same speciation rate as other areas, but a significantly lower extinction rate (Supplementary Fig. S6; and Supplementary Appendix 7B, available on Dryad). This meets the expectations found in other studies of montane habitats having lower extinction rates than non-montane habitats (Colwell et al. 2008; Loarie et al. 2008, 2009), but is contrary to that found for diversification of Ericaceae into montane habitats where a higher speciation rate

was found (Schwery et al. 2015). It should be noted that gesneriad lineages are suggested to have been in western South America/the Andes as early as approximately 76 Ma, well before most of the Andean uplift, and even those more recent diversifications into the Andes (e.g., Columneinae) occurred around 17 Ma, possibly corresponding to the main period of central Andes uplift (Hoorn et al. 2010). This greatly predates many of the commonly discussed "adaptive radiations" into the Andes, such as *Lupinus* which diversified into the Andes approximately 3 Ma (Drummond et al. 2012).

Pacific *Cyrtandra* is a lineage that could provide an example of significant geographic influence on diversification rates with more detailed study. We are not able to directly test this idea here given the sampling available, in part because to test for influence of geographic area on diversification using GeoSSE, there has to be at least one species that is found in both the focal area and outside the focal area. As we currently understand the diversity of *Cyrtandra*, only a single lineage has dispersed into the Pacific and there are no species that straddle this boundary (Atkins et al. 2001; Clark et al. 2008, 2009, 2013); however, the boundary areas separating Southeast Asia and the Pacific are the poorest explored for *Cyrtandra* diversity (Papua New Guinea, Solomon Islands, and vicinity). Ongoing and future work to better understand the geographical distribution of and diversification patterns of *Cyrtandra* may provide a compelling example of geography-driven increased diversification rates (H. Atkins, ongoing; J. R. Clark, ongoing). A number of other factors could be influencing diversification rates in the clade, including hybridization (Smith et al. 1996; De Villiers et al. 2013) and the interaction of avian frugivores and fleshy fruits in the island forest understory (Givnish et al. 1995; Givnish 2010; Theim et al. 2014). Additionally, appropriateness of analytical approaches need to be further considered as GeoSSE is a DEC-like model and therefore leaves out founder-event speciation. Founder events are likely very important for groups like *Cyrtandra* where lineages are distributed across island systems, but species are predominantly narrowly endemic (Price and Wagner 2004; Clark et al. 2009). Whether driven by invasion of the Pacific or one or more of these other as yet unstudied characters, it is clear that Pacific *Cyrtandra* represents one of the most striking diversification rate increases in Gesneriaceae (Table 2; Fig. 3).

Beslerieae provides an interesting case of unclear diversification processes and no clear hypothesis of what characteristics might be involved in the diversification patterns we see. Core Beslerieae are inferred to have an elevated speciation rate over background rates (0.6466491 vs. 0.2183877), and also a significantly elevated extinction rate relative to background (0.3916895 vs. 0.0584205; Table 2; Fig. 3). This clade demonstrates interesting variation in growth form from small rosette plants to moderate-sized shrubs, and substantial variation in floral form (Wiehler 1975; Skog and Kvist 2000; Roalson and Clark 2006). The inferred high speciation rate in this clade is contrary to the expectation for generally woody lineages which are typically found to have fewer species, and slower diversification rates, than herbaceous lineages (Ricklefs and Renner 1994; Dodd et al. 1999; Smith and Donoghue 2008; Smith and Beaulieu 2009). Although Beslerieae has a large number of floral forms, many of which are likely associated with hummingbird pollination, that is no different from much of the rest of the Gesnerioideae diversity (Fig. 3; Supplementary Fig. S7, available on Dryad).

Floral Drivers of Diversification Rates

Gesneriaceae is renowned for their floral diversity, and floral adaptation to pollinators and/or frequent change of pollinator among closely related species has been commonly invoked as a driver of diversification (Wiehler 1976; Roalson et al. 2003; Perret et al. 2007). However, this has not been tested explicitly until now. Our analyses suggest that in New World Gesnerioideae, pollination syndrome and one of its primary underlying characters, primary flower color, are having a significant influence on diversification rates (Fig. 2; Supplementary Figs. S7 and S8; and Supplementary Appendix 11, available on Dryad). Our model fitting would suggest that this rate shift is through an increase in speciation rates, not a change to extinction rate probabilities (although extinction probabilities are notoriously difficult to estimate with confidence; see discussion in Rabosky 2010). This is also supported by the significant effects of flower shape, flower color, and flower gibbosity on Pagel's *K* (trait evolution at speciation) for the Gesnerioideae. Although ornithophilous flowers are also found in the Old World (predominantly in *Aeschynanthus* and *Agalmyla*), Old World ornithophily does not appear to be directly influencing diversification rates. Studies in other Old World ornithophilous lineages (*Hakea*, Proteaceae) have suggested a different kind of dynamic in the probability of change between bird and insect pollination, possibly associated with the differences between bird pollinators in the Old World and hummingbirds (Mast et al. 2012). Studies in cacti have also suggested dynamic transitions among pollination syndromes, including ornithophily, with transitions to specialized pollination syndromes from bee pollination associated with increased diversification rates (Hernández-Hernández et al. 2014). However, that study suggested a linkage between growth form, pollination syndrome, and diversification rate change, and which of these factors (or others) are the proximal drivers of rate shifts remain unclear. While not apparently associated with hummingbird pollination, there is significant evolution of flower color and shape at speciation in Didymocarpoideae (as measured by Pagel's *K*; Supplementary Appendix 10, available on Dryad); however, our understanding of pollinators of Old World Gesneriaceae is rather incomplete and this finding needs to be further explored.

As with other New World lineages with ornithophilous species (see review in Givnish 2010),

previous authors have invoked the influence of hummingbird diversification on diversification rates of Gesneriaceae (Wiehler 1976; Perret et al. 2013). The age of the most-recent common ancestor of extant hummingbirds is 22.4 Ma (20.3–24.7, 95% HPD; McGuire et al. 2014). This closely approximates the origins of the clades dominated by hummingbird-pollinated species in Gesnerioideae—crown Columneinae: 22.40, crown Beslerieae: 22.62, and stem Ligeriinae: 25.65, and crown Ligeriinae: 15.17 (Fig. 1; Supplementary Appendix 6, available on Dryad). Given the concurrent timing of diversification of these clades and hummingbirds, the strong evidence for increased diversification rates of two of these predominantly hummingbird-pollinated clades (Columneinae and Beslerieae; Table 2), and the strong effect of ornithophily on diversification rates in the Gesnerioideae (Supplementary Appendix 11, available on Dryad), we would suggest that there is strong evidence that hummingbird pollination is a significant driver of diversification in Columneinae and Beslerieae. It should be noted that within Columneinae there are two significant rate shifts: core Columneinae, which is predominantly distributed from the Andes north into Central America and the Caribbean, and core *Nematanthus*, which is predominantly distributed in the Atlantic coastal forests of Brazil. Both of these areas have high hummingbird diversity that quite likely has driven significant components of lineage diversification in these clades. It should be noted, though, that demonstrating a cause-and-effect relationship of these characters and diversification rates is not possible with the data and analyses presented here, but our work here is instead supportive of the hypothesis that the association of floral diversification with hummingbird pollination is a significant contributor to increase in diversification rates in these lineages.

Although there appears to be hummingbird-associated diversification rate increases in the aforementioned clades, there are hummingbird-pollinated species distributed throughout Gesnerioideae (Supplementary Fig. S7, available on Dryad). Why are diversification rate increases not found in these other lineages? One possible answer is that there is a rate increase associated with availability of hummingbird pollinators, but it is just a more subtle shift. We note that the core Gesnerioideae diversification rate (0.25479) is elevated over the core Gesnerioideae background rate (0.13140; Table 2). This could be indicative of a general rate shift associated with availability of hummingbird pollinators. There is considerable pollinator variability within most of the Gesnerioideae clades, and this variability has been suggested to influence lineage splitting in a number of these groups (e.g., *Achimenes*, Roalson et al. 2003; *Sinningia*, Perret et al. 2007). Further, some of these clades have primarily or exclusively diversified in the Caribbean Islands (e.g., Gesneriinae). It is notable that unlike some other lineages which have been suggested to have undergone adaptive radiations in the Caribbean (e.g., *Anolis* lizards, Losos et al. 1998; Revell et al. 2012), we do not find significant evidence here for changes in diversification rates in Gesneriinae (non-significant in BAMM analysis using Bayes factors) despite significant variation in pollination syndromes among species and edaphic specialization (Skog 1976; Martén-Rodríguez et al. 2009, 2010; Martén-Rodríguez and Fenster 2010). Abrahamczyk et al. (2015) suggested that this could be due to few opportunities for geographical isolation on small islands when lineages are predominantly bird pollinated, as hummingbirds can effectively move pollen over large distances. This needs to be tested further in Gesneriinae.

Growth Form Drivers of Diversification Rates

We here address the potential impact of two growth forms on diversification rates: 1) epiphytism and 2) unifoliate growth, as found in *Streptocarpus* (Möller and Cronk 2001; Nishii et al. 2004; Harrison et al. 2005), and also found in other Old World lineages (e.g., *Monophyllaea*; Cronk and Möller 1997; Imaichi et al. 2001). Our analyses provide somewhat mixed support for epiphytism-driven diversification rate increases in the Gesnerioideae. Epiphytism is clearly associated with two of the clades found by BAMM to have elevated diversification rates (Table 2; Fig. 3). However, as has been noted recently (Rabosky and Goldberg 2015), attributing cause-and-effect to BiSSE results alone can be problematic for a number of reasons, including problems with high Type I error rates under some circumstances. The fact that quite different models are chosen as best fit by BiSSE on the whole Gesneriaceae phylogeny and the Gesnerioideae subtree likely indicates that this test is particularly sensitive to the distribution of states and tree shape, factors suggested to be important by Rabosky and Goldberg (2015). This is not what we see when we look at the same comparisons for ornithophyly (see discussion above), where the same model is chosen for the whole tree and Gesnerioideae subtree BiSSE analyses (Supplementary Appendix 11D–E, available on Dryad). The importance of epiphytism in diversification rates is additionally suspect given that the best-fit model suggests that epiphytes have the same speciation rate as non-epiphytes, but have a much lower extinction rate. This is contrary to what has been typically proposed for epiphytes in other lineages (Bromeliaceae, Givnish et al. 2014; Orchidaceae, Gravendeel et al. 2004; Silvera et al. 2009; Givnish et al. 2015). This also could be a case where there is entanglement of epiphytism with other characters not measured here, and it is not epiphytism per se that is driving diversification rate changes, but other correlated characters. For instance, Weber (2004) suggests that there is a strong correlation between the epiphytic habit and ornithocory (dispersal of seeds by birds). In orchids, epiphytism is also often correlated with CAM photosynthesis (Silvera et al. 2009; Givnish et al. 2015), which is a condition generally not developed, or at least not considered to be prevalent, in Gesneriaceae. This is

an area that needs more study as in a study of two Old World and two New World epiphytic gesneriads, one of the New World species (*Codonanthe crassifolia*) was found to have some CAM-like characteristics (CAM-cycling and CAM-idling; Guralnick et al. 1986). Whether there is more prevalence of CAM or CAM-like photosynthetic mechanisms in epiphytic gesneriads, and what role this might play in the diversification pattern requires further study. It should be noted that the pattern found here of having high predicted diversification rates of epiphytes in both the Andes and the Atlantic Forest of Brazil is also found in Bromeliaceae (Givnish et al. 2014). The high diversification rates for core Columneinae and core *Nematanthus* in the Andes and Atlantic Forests, whether driven by epiphytism or not, adds another example to these two areas of high diversification rates in plants (Jansson and Davies 2008).

Modeling the influence of the unifoliate growth form on diversification rates strongly suggests that this growth form positively influences speciation rate (Supplementary Fig. S8; and Supplementary Appendix 11, available on Dryad). Previous work on the evolution of growth forms in *Streptocarpus* supported multiple transitions in form, particularly between rosulate and unifoliate growth (Möller and Cronk 2001). Although it is yet unclear how unifoliate growth might influence diversification rates, previous authors have suggested that this is an adaptation for deep shade (Möller and Cronk 2001). Unifoliate growth is found outside of the *Streptocarpus* clade (particularly in *Monophyllaea*), but there is no support for diversification rate changes in these other unifoliate lineages (Fig. 3). The results presented here on diversification rates associated with unifoliate growth in *Streptocarpus* might in reality reflect the influence of transitions among the three growth forms found in this clade, rather than the influence only of unifoliate growth (Möller and Cronk 2001). Further, there are some data to suggest that hybridization might be particularly influencing the diversification of the unifoliate and rosulate *Streptocarpus*, and this merits more detailed investigation (De Villiers et al. 2013).

Conclusions

We have here demonstrated complex interactions of diversification rates, floral morphology, vegetative morphology, and geography. Background diversification rates in Gesnerioideae are substantially higher than those in Didymocarpoideae, and background rates and elevated clade-specific rates across the family have very different rate characteristics (Fig. 4), suggesting that while there are both Old World and New World clades with elevated diversification rates, the processes driving these rate changes are substantially different. Although characters like pollination syndrome have been previously suggested to influence diversification in Gesneriaceae (Wiehler 1976; Roalson et al. 2003; Perret et al. 2013), this is the first explicit test to demonstrate where in the phylogeny this and other characteristics are influencing rates of diversification. It is clear that ornithophily is important to diversification rate increases in the New World, despite also occurring in the Old World. If epiphytism is involved in increased diversification rates, it is likely important in conjunction with ornithophily and geography (e.g., Andean or Brazilian Atlantic Forest distributions). This is essentially the same set of interacting factors suggested by Givnish et al. (2014) to be driving diversification rate changes in Bromeliaceae. Unlike gesneriads and bromeliads, orchids have been little influenced by ornithophily, and demonstrate similar diversification rate changes associated with geography (particularly mountainous areas) and epiphytism (Givnish et al. 2015).

Unifoliate growth is apparently increasing diversification rate of the *Streptocarpus* lineage, and, while we were not able to explicitly test the root cause, Pacific *Cyrtandra* has undergone a significant diversification rate increase, possibly driven by the invasion of the Pacific, and also possibly associated with other characteristics of that lineage. It should be noted that with all of these characteristics, it is quite possible that the underlying trait influencing diversification rates is not the particular character states we have explored here, but instead could be the "evolvability" or plasticity in these traits or others in these clades that is influencing diversification rates (Rabosky 2012). Further work will be necessary to explore the interplay of specific character states and the possible "evolvability" of those traits on diversification rates. In all, there have been at least five significant upward shifts in diversification rates in Gesneriaceae, each with different hypothesized underlying character and geographic drivers. The mega-phylogenetic hypothesis presented here further refines our understanding of lineage relationships in Gesneriaceae and highlights the need to further explore the potential drivers of diversification at finer geographic scales, across a broader selection of morphological and physiological traits, and with more detailed species sampling. Although there are many possible predictors of diversification dynamics (most as yet untested), we are starting to see a confluence of patterns at some scales, as found among gesneriads, bromeliads, and orchids. Whether other shared characters and geographic patterns will be found at other phylogenetic scales is yet to be seen, but the current rapid advancement of comparative methods (e.g., Rabosky and Huang forthcoming) should only continue to increase our abilities to test for the drivers of diversification dynamics within and across lineages.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.1br13.

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